DETECTION OF PORCINE ADULTERATION IN COSMETIC CREAM FORMULATION VIA TAQMAN PROBE REAL-TIME POLYMERASE CHAIN REACTION

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By

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Halal authentication in the cosmetic cream is relatively a new study. Biomolecular approach is said to be efficient and effective for the detection of species-specific target. However, the complex matrices that came from the variety composition of the ingredients and the harsh process have become challenges for the detection of species-specific targeting porcine. This challenges are due to the fragmentation of high molecular weight DNA (Deoxyribonucleic acid) into a smaller fragment and high possibility of denaturation of DNA. The challenges in detection become more significance as there are ingredients that are potentially can inhibit the Polymerase Chain Reaction (PCR) to be happened. Ethylenediaminetetraacetic acid (EDTA) is one the common ingredients that are used in the cosmetic cream are found can inhibit the PCR reaction due to its chelates properties. This aim of this research is to study the on the Halal detection targeting porcine as the species-specific in cosmetic cream using real time PCR (qPCR) as a detection tool. Hence, the laboratory prepared cream was formulated with the addition of the 10% (w/w) EDTA. A part from that, lard was spiked into the prepared cream ranging from 1%, 3% and 5% (w/w). A modified Cetyl trimethylammonium bromide (CTAB) method was developed that encompassed on pre-treatment sample and purification method and successfully extracted the DNA. These qPCR assays were subjected to the 1%, 3% and 5% of lard containing cream samples providing their Cq value respectively. Universal primer 18s rRNA TaqMan qPCR was employed. This assay had amplified all the samples at 187 bp at the Cq value of 33.49±0.08, 32.31±0.11 and 23.49±0.17 contributing that eukaryotic DNA was successfully extracted from the prepared cream. Hence further analysis employing TaqMan qPCR was done with the porcine-specific primer involving MPRE (nuclear DNA) and Cyt b gene (mitochondrial DNA). These two primers were utilized as a sensitivity comparison study between the nuclear DNA and mitochondrial DNA. Cyt b had successfully amplified the samples at 109 bp at the Cq value of 35.89±0.55,

36.78±0.38 and 24.14±0.19 while there was negative amplification of the MPRE. The porcine commercial cream branding ROREC that contained porcine derivatives was tested to detect the presence of porcine. Universal TaqMan qPCR assay was found to amplify this commercial as positive amplification with the Cq value of 27.45±0.25 while there was no amplification occurred at both MPRE and Cyt b. Justification was made to this outcome.The amplified amplicon from the Universal assay was cloned to generate the sequence. No porcine DNA was traced from the sequence using the blast tool. This Halal authentication for the cosmetic cream on the other hand has become a great challenge to deal with as the fragmented DNA or too low amount of porcine DNA from the sample.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGESANAN CAMPURAN BABI DALAM FORMULASI KRIM KOSMETIK MELALUI MASA NYATA TINDAKAN RANTAIAN POLIMERASE TAQMAN PROB

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Pengesanan halal dalam krim kosmetik adalah kajian yang agak baru. Pendekatan Biomolekul untuk mengesan sasaran spesifik-spesies adalah cekap dan berkesan. Walaubagaimanapun, matrik kosmetik yang komples yang diperbuat daripada pelbagai komposisi bahan-bahan dan proses yang rumit telah menjadi cabaran untuk pengesanan spesifik-spesies babi. Cabaran ini adalah disebabkan oleh fragmentasi berat molekul tinggi Asid Deoksiribonukleik (DNA) kepada serpihan yang lebih kecil dan tinggi kemungkinan denaturasi DNA. Cabaran dalam pengesanan menjadi lebih terkesan kerana terdapat ramuan berpotensi yang boleh menghalang Reaksi Rantaian Polimerase (PCR) untuk berlaku. Asid Ethylenediaminetetraasetik (EDTA) adalah salah satu bahan yang biasa digunakan dalam krim kosmetik dan dianggap boleh menghalang reaksi PCR kerana bersifat chelate. Matlamat penyelidikan ini adalah untuk mengkaji pengesanan halal yang menyasarkan babi sebagai spesifik-spesies dalam krim kosmetik menggunakan Masa Nyata Reaksi Raintaian Polimerase (qPCR) sebagai alat pengesanan. Oleh itu, kirm yang disediakan di makmal telas dirumus dengan penambahan 10% (b/b) EDTA. Selain itu, lemak babi telah ditambah ke dalam krim yang disediakan dengan berkadaran 1%, 3% dan 5% (b/b). Kaedah Cetyl Trimethylammonium Bromida (CTAB) terubahsuai telah dikembangkan yang merangkumi kaedah pra-rawatan dan kaedah penulenan sampel. Kaedah yang berkesan telah dibangunkan bagi mendapatkan produk PCR yang berkualiti tinggi dan produk yang boleh disalinkan oleh sampel dan kaedah ini berjaya dibangunkan dengan mengekstrak keluar DNA. Semua cerakin qPCR adalah tertakluk kepada 1%, 3% dan 5% kandungan lemak babi di dalam krim yang memberikan nilai Cq secara berturutan. Universal 18s rRNA TaqMan qPCR telah digunakan. Cerakin ini telah menyalin kesemua sampel pada 187 bp pada Cq 33.49±0.08, 32.31±0.11 dan 23.49±0.17 dengan menyumbang bahawa DNA eukariot telah berjaya diekstrak dari krim yang disediakan. Oleh itu, analisis selanjutnya menggunakan TaqMan qPCR telah dilakukan dengan gen babi yang spesifik iaitu MPRE (DNA nuklear) dan Cyt b gen

(DNA mitokondria). Kedua-dua gen ini digunakan sebagai kajian perbandingan sensitiviti anatra DNA nuclear dan DNA mitokondria. Gen Cyt b telah berjaya disalinkan sampel pada 109 bp manakala pada nilai Cq 35.89±0.55, 36.78±0.38 dan 24.14±0.19, manakala terdapat penyalinan negatif bagi MPRE. Satu krim komersial babi berjenama ROREC yang mengandungi bahan terbitan babi diuji untuk mengesan kehadiran spesies ini. Pengujian qPCR Universal TaqMan didapati menyalin dengan salinan positif pada nilai Cq 27.45±0.25 manakala tidak terdapat penyalinan pada kedua-dua MPRE dan Cyt b. Justifikasi telah dilakukan bagi hasil keputusan ini. Amplikon yang disalin dari cerakin Universal telah diklon untuk menjana urutan DNA. Tiada DNA babi dikesan dari urutan menggunakan BLAST. Pengesahan Halal untuk krim kosmetik sebaliknya telah menjadi cabaran besar untuk dikesan kerana struktur DNA yang berpecah atau jumlah DNA babi yang terlalu sedikit dari sampel.

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
bp	base pair
CTAB	Cetyl trimethylammonium bromide
Cq	Cycle quantification
Cyt b	Cytochrome b
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EtBr	Ethidium Bromide
FDA	Food and Drug Administration
FTIR	Fourier-transform Infrared Spectroscopy
GMO	Genetic Modified Organism
g	gram
GRAS	Generally Recognized as Safe
kDA	kilo Dalton
LOD	Limit of Detection
MWCO	Molecular Weight Cut Off
mg	milligram
mM	millimeter
mL	milliliter
MPRE	Meat Animal Research Centre Porcine Repetitive Element
mt DNA	mitochondrial DNA
NCBI	National Centre for Biotechnology Information
nM	nanometer
NMWL	Nominal Molecular Weight Limit
NTC	No Template Control
o/w	oil in water

PCR	Polymerase Chain Reaction
qPCR	real-time Polymerase Chain Reaction
rpm	revolutions per minute
rRNA	ribosomal ribonucleic acid
TE	Tris-EDTA
w/o	water in oil
w/w	weight over weight
μL	microliter

CHAPTER 1

INTRODUCTION

1.1 Research background

The existence of cosmetics has been around for a long time for its notorious function. The main purpose for using cosmetics is to enhance attractiveness, protect skin and hair and prevent aging (Mitsui, 1997). Cosmetics products are enjoyed by millions of people all over the world. The cosmetic industry is constantly changing with the new distinctive product and is introduced at the international level that has set as a trend for other cosmetics manufacturer (Mohd Daud, Abdul Aziz, Baharudin, & Shamsudin, 2012). As the consequences from the advance in cosmetic innovations, consumers often do not know the source of ingredients and its processing. The identity of ingredients in processed products or composite mixtures is not usually readily apparent. Thus, verification of authentication of the sources to the consumer may be required (Lockley & Bardsley, 2000).

Halal is attaining its attention globally due to the awareness in Halal food and Halal product. Muslim consumers especially have broadened their perspective of halal from meat-based to wide range of products. This overview has brought Muslim in seeking Halal integrity not only from food but also in beverages, insurance, leather product, pharmaceutical and even in cosmeceutical field (Murray, 2012). It is reported that Muslim population is growing tremendously in a total of nearly 2 billion. Malaysia, a country that has more than 60% of Muslims population, is a market provider and halal hub centers for the worldwide market (Kaur, 2018: Iberahim, Kamaruddin, & Shabudin, 2012).

In recent years, the emerging halal cosmetic has created an awareness among muslim and non-muslim consumers worldwide. It is known that Halal cosmetic which includes body and skin care products are free from any materials that are forbidden by the islamic guidelines. Halal cosmetic is considered as an innovation to the cosmetic industry as it introduces new external and internal operations that will serve the growing customer needs. Halal requirement in cosmetics is driven by ingredients that are in many cases not Halal (have pork or pork by-products) and this is a concern to many Muslim customers (Yeo, Hj, Mohamed, & Mohd, 2018). Thus, the detection of the presence of porcine or porcine derivaties plays the significance role for the Muslim consumer.

The manufacturing of cosmetic can be classified as a highly processed product that urdergone harsh process. These highly processed products are manufactured from ingredients of animal or plant origins, which undergo various and usually multiple treatments either chemical or physical, commercially processed, and used as ingredients in the cosmetic, food and other industries (Hashim & Mat Hashim, 2013). Nanotechnology for instance has grown in both pharmaceutical and cosmeceutical.

Nanoemulsion is one of the applications of nanotechnology in cosmetic which involves oil in water (o/w) emulsion that uses high pressure homogenizer to produce emulsion of extremely low particels size up to nanometer (nm) (Sharma & Sarangdevot, 2012; Yukuyama *et al.*, 2016). This technique is widely used in cosmetic product to provide a better penetration into the skin.

On the other hand, there are approaches that can detect lard in the wide range of processed food. The developed methods are either lipid-based method, protein-based method or DNA-based method. These developed methods can determine the adulteration of porcine or its derivative in food products such as chocolate, meatball, sausages and nugget. Since cosmetic is also classified as processed product, an analytical method in detection of lard in formulated cosmetic lotion also has been developed. This analytical method is using FTIR spectroscopy to determine emulsifying system of virgin coconut oil in cream formulation (Lukitaningsih, Sa'adah, Purwanto, & Rohman, 2012). However this method might have limitation on lard detection of commercial nanotechnology cosmeceutical product. Since FTIR spectroscopy can only be used for certain formulations (Rohman & Man, 2011) due to the cosmetic physical properties difference.

Lard composes of a fat tissue and the DNA trace may reside in the tissue structure. Thus, Polymerase Chain Reaction (PCR) techniques is being utilized for the PCR analysis targeting specific genes for both nuclear and mitochondrial DNA genes. This DNA-based approach is a superior technique as it is able to feasibly detect the lard constituent in cosmetic cream formulation as PCR is well known as the specific, reproducible, sensitive, rapid processing time and low cost (Alfie & Farouk, 2013) technique. Its ability to amplify at the targeted sequence assists in detection of interest region of DNA. Plus, PCR is proven to be an adequate for detection of small amount of DNA in a rapid and sensitive manner (Aida, Man, Wong, Raha, & Son, 2005). Real time PCR is another adaptation method of PCR that provides speed in result as this method uses fluorescence detection system. This detection system data can be directly obtained by avoiding electrophoresis and allowing the selection for short sequences for amplification (López-Andreo, Lugo, Garrido-Pertierra, Prieto, & Puyet, 2005). In addition, real-time PCR has been increasingly used for fast, sensitive and specific detection of species-specific DNA in a variety of processed products (Cai, Gu, Scanlan, Ramatlapeng, & Lively, 2012).

1.2 Problem statement

The potential detection of porcine in cosmetic product in halal authentication using molecular approach is not fully discovered which gives novelty to this study. In addition, the source of the ingredients of the cosmetic product is not known for the Halal authentication which provides doubtful especially for the Muslim consumer.

1.3 Study objectives

i. To evaluate porcine specific targeting nuclear DNA (MPRE) and mitochondrial DNA (Cyt b) via TaqMan probe Real-Time PCR assay.
ii. To detect the porcine presence in formulated-lard cream using nuclear DNA (MPRE) and mitochondrial DNA (cyt b) via TaqMan probe Real-Time PCR assay.
iii. To make a comparative study on the sensitivity and specificity between the nuclear and mitochondrial DNA.

1.4 Scope of the study

In this study, the DNA extraction is relatively dependent to the physical properties of the cream. Plus, the differences in physical properties and ingredients in each commercial cosmetic cream has lead to a limitation to detect to only one type of real sample of commercial porcine cream. Whilst, EDTA is the most common ingredient in the cosmetic making aside from the others that used was mainly studied due to its chelates properties that can inhibit PCR assay.

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